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Interplay between Protective and Inhibitory Antibodies Dictates the Outcome of Experimentally Disseminated Candidiasis in Recipients of a *Candida albicans* Vaccine
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Measurement of T-Cell-Derived Antigen Binding Molecules and Immunoglobulin G Specific to *Candida albicans* Mannan in Sera of Patients with Recurrent Vulvovaginal Candidiasis
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Current Status of Nonculture Methods for Diagnosis of Invasive Fungal Infections
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Immunological Characterization of Asp f 2, a Major Allergen from *Aspergillus fumigatus* Associated with Allergic Bronchopulmonary Aspergillosis
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- ☐ Brahim El Moudni, Marie-Helene Rodier, Gyslaine Daniault, and Jean Louis Jacquemin
Improved Immunodiagnosis of Human Candidiasis by an Enzyme-Linked Immunosorbent Assay Using a *Candida albicans* 52-Kilodalton Metallopeptidase
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Genetically distinct strains of *Candida albicans* with elevated secretory proteinase production are associated with diarrhoea in hospitalized children.
J Gastroenterol Hepatol. 2000 Jan;15(1):53-60.
PMID: 10719748 [PubMed - indexed for MEDLINE]

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Related Articles, Links

The genotypic relationship of *Candida albicans* strains isolated from the oral cavity of patients with denture stomatitis.
J Med Microbiol. 1995 May;42(5):372-9.
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Immunol Cell Biol. 1995 Apr;73(2):125-33.
PMID: 7797232 [PubMed - indexed for MEDLINE]

☐ 6: Franklyn KM, Warmington JR.

Related Articles, Links

The expression of *Candida albicans* enolase is not heat shock inducible.
FEMS Microbiol Lett. 1994 May 15;118(3):219-25.
PMID: 8020745 [PubMed - indexed for MEDLINE]

☐ 7: Costantino PJ, Franklyn KM, Gare NF, Warmington JR.

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PMID: 7907579 [PubMed - indexed for MEDLINE]

- ☐ **8:** [Calderone R, Diamond R, Senet JM, Warmington J, Filler S, Edwards JE.](#) Related Articles, Links
 Host cell-fungal cell interactions.
 J Med Vet Mycol. 1994;32 Suppl 1:151-68. Review. No abstract available.
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 Cloning and nucleotide sequence analysis of the Candida albicans enolase gene.
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 PMID: 8359671 [PubMed - indexed for MEDLINE]
- ☐ **10:** [Franklyn KM, Warmington JR, Ott AK, Ashman RB.](#) Related Articles, Links
 An immunodominant antigen of Candida albicans shows homology to the enzyme enolase.
 Immunol Cell Biol. 1990 Jun;68 (Pt 3):173-8.
 PMID: 2228032 [PubMed - indexed for MEDLINE]
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TITLE: Cytoplasmic antigens of candida albicans and methods of using the same

DATE-ISSUED: February 21, 1989

INVENTOR-INFORMATION:

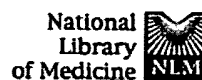
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Strockbine; Nancy A.	Bethesda	MD		

US-CL-CURRENT: 435/7.31; 435/70.21, 436/518, 436/548, 530/371, 530/388.5

CLAIMS:

What is claimed is:

1. A diagnostic method for disseminated or invasive candidiasis comprising:
 - a. contacting blood serum with a composition containing a substantially biochemically pure preparation of a cytoplasmic antigen of *C. albicans*, said antigen having an apparent molecular weight selected from the group consisting of 48-52 Kd, 35-38 Kd and 120-135 Kd, said antigen being detectable in humans during disseminated candidiasis but not during non-invasive *C. albicans* infections; and
 - b. detecting antibody bound by the antigen of said preparation.
2. A diagnostic method according to claim 1 wherein the detecting means is selected from the group consisting of latex agglutination, radioimmunoassay, enzyme-linked immunosorbent assay, and immunoblot assay.
3. A diagnostic method according to claim 1 wherein the antigen is the 48-52 Kd antigen.
4. A diagnostic method according to claim 1 wherein the antigen is recognized by monoclonal antibody produced by a hybridoma selected from the group of hybridomas consisting of ATCC #HB-8397 and ATCC #HB-8398.
5. A substantially biochemically pure preparation of a cytoplasmic antigen of *C. albicans*, said antigen having an apparent molecular weight selected from the group consisting of 48-52 Kd, 35-38 Kd and 120-135 Kd, said antigen being detectable in humans during disseminated candidiasis but not during non-invasive *C. albicans* infections.
6. A preparation according to claim 5 of the 48-52 Kd antigen.
7. A substantially biochemically pure preparation of a cytoplasmic antigen of *C. albicans* which is recognized by a monoclonal antibody produced by a hybridoma selected from the group of hybridomas consisting of ATCC #HB-8397 and ATCC #HB-8398.



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Production of antibodies to antigens of *Candida albicans* in CBA/H mice.

Costantino PJ, Franklyn KM, Gare NF, Warmington JR.

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MeSH Terms:

- Animal
- Antibodies, Fungal/biosynthesis*
- Antigens, Fungal/immunology*
- CD4-Positive T-Lymphocytes/immunology
- *Candida albicans*/immunology*
- Female
- Heat-Shock Proteins/immunology
- Mice
- Mice, Inbred CBA
- Precipitin Tests
- Support, Non-U.S. Gov't

Substances:

- Heat-Shock Proteins

Infect. Immun., 04 1994, 1400-1405, Vol 62, No. 4
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Production of antibodies to antigens of *Candida albicans* in CBA/H mice

PJ Costantino, KM Franklyn, NF Gare and JR Warmington

School of Biomedical Sciences, Curtin University of Technology, Bentley, Perth,
Australia.

Reported targets of the specific immune responses to *Candida albicans* in human candidiasis include a 47-kDa breakdown product of a 90-kDa heat shock protein (HSP 90) (R. Matthews and J. Burnie, FEMS Microbiol. Lett. 60:25-30, 1989) and the 48-kDa enolase (K.M. Franklyn, J.R. Warmington, A.K. Ott, and R.B. Ashman, Immunol. Cell Biol. 68:173-178, 1990). These proteins are immunodominant antigens of *C. albicans*. Western blotting (immunoblotting) and immunoprecipitation were used to investigate the humoral response in a mouse model of systemic candidiasis. Resolution of systemic candidiasis in CBA/H mice is associated with a high level of antibody reactivity to *C. albicans* antigens. A significant antibody response against a non-HSP antigen of 96 kDa which was distinct from the *C. albicans* HSP 90 antigen was detected. Significant antibody reactivity against an HSP of 75 kDa was also detected. We concluded that resolution of *C. albicans* infections in CBA/H mice was associated with antibodies to an HSP and a non-HSP of 75 and 96 kDa, respectively.

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- Chaffin, W. L., Lopez-Ribot, J. L., Casanova, M., Gozalbo, D., Martinez, J. P. (1998). Cell Wall and Secreted Proteins of *Candida albicans*: Identification, Function, and Expression. *Microbiol Mol Biol Rev* 62: 130-180 [[Abstract](#)] [[Full Text](#)]
- Bromuro, C., La Valle, R., Sandini, S., Urbani, F., Ausiello, C. M., Morelli, L., Fe d'ostiani, C., Romani, L., Cassone, A. (1998). A 70-Kilodalton Recombinant Heat Shock Protein of *Candida albicans* Is Highly Immunogenic and Enhances Systemic Murine Candidiasis. *Infect. Immun.* 66: 2154-2162 [[Abstract](#)] [[Full Text](#)]



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Identification of *Candida albicans* antigens reactive with immunoglobulin E antibody of human sera

A Ishiguro, M Homma, S Torii and K Tanaka

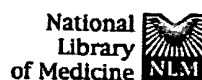
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Candida albicans antigens which reacted with immunoglobulin E (IgE) antibodies of 57 allergic patients were detected by immunoblotting. Of the various antigens, the 175-, 125-, 46-, 43-, and 37-kDa antigenic components reacted most frequently with the patient sera. To purify the major antigens, *C. albicans* cells were fractionated. The 46-, 43-, and 37-kDa antigens were recovered in cytoplasmic fractions, but the 175- and 125-kDa antigens were not recovered in any fraction. The 46-, 43-, and 37-kDa antigens were purified from cytoplasmic fractions by DEAE and P11 ion-exchange chromatography. Antigens were isolated by cutting bands out of sodium dodecyl sulfate-polyacrylamide gels. The purified components confirmed by immunoblotting were next processed for amino acid sequencing. Parts of the sequences of the 46-, 43-, and 37-kDa antigens had significant levels of homology with *Saccharomyces cerevisiae* glycolytic enzyme enolase, phosphoglycerate kinase, and aldolase, respectively. Rabbit IgG antibodies prepared against the 46- and 43-kDa antigens strongly cross-reacted with the homologous proteins of *S. cerevisiae*. However, *S. cerevisiae* enolase and phosphoglycerate kinase did not cross-react with IgE of patient sera. This result suggests that IgE antibodies against only small parts of their epitopes are elevated in the allergic patients. Since enolase is reported to be a major antigen for systemic candidiasis, this enzyme may be the immunodominant protein in both allergies and fungal infections.

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- Gozalbo, D., Gil-Navarro, I., Azorin, I., Renau-Piqueras, J., Martinez, J. P., Gil, M. L. (1998). The Cell Wall-Associated Glyceraldehyde-3-Phosphate Dehydrogenase of *Candida albicans* Is Also a Fibronectin and Laminin Binding Protein. *Infect. Immun.* 66: 2052-2059 [\[Abstract\]](#) [\[Full Text\]](#)



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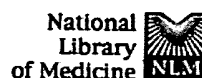
An isoprotein of enolase from the yeast *Saccharomyces cerevisiae* was reported to be a heat shock protein. The possible role of the *C. albicans* enolase as a heat shock protein was therefore investigated. The de novo synthesis of *C. albicans* enolase protein and mRNA did not increase during heat stress, but remained constitutively expressed. Amino acid similarity to the heat shock proteins suggests that although the *C. albicans* enolase is not a classical heat shock protein, it may be a member of a group of constitutively expressed, structurally related proteins, the heat shock cognate proteins.

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Applied Biochemistry**Purification of native enolase from medically important *Candida* species.****Ballantyne DS, Warmington JR.**

School of Biomedical Sciences, Curtin University of Technology, GPO Box U1987, Perth WA 6845, Australia.

The 48 kDa glycolytic enzyme, enolase, has been identified as an immunodominant antigen in *Candida albicans* infections. It has also been identified as an important fungal allergen. Enolase from a number of medically important *Candida* species has been purified using a two-step anion- and cation-exchange chromatography method that was preceded by an organic extraction. The enolases purified by this method have a high specific activity and the procedure is 40% efficient, with an average of 5 mg of enolase/g of *Candida* cells. The purification of native enolase from medically important *Candida* species will enable the immunological significance and interspecies relationships of this major fungal antigen to be investigated.

PMID: 10814591 [PubMed - indexed for MEDLINE]

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- ☐ 1. 6218129. 15 May 98; 17 Apr 01. Inflammatory bowel disease first step assay system. Walsh; Michael J., et al. 435/7.21; 435/7.24 435/7.31 435/7.95 436/506 436/513. G01N033/564.
- ☐ 2. 6121420. 13 Apr 99; 19 Sep 00. Diagnosis of fungal infections, and a chitin-binding lectin useful in such diagnoses. Laine; Roger A.. 530/350; 435/7.31. C07K014/28.
- ☐ 3. 5968741. 11 Apr 97; 19 Oct 99. Methods of diagnosing a medically resistant clinical subtype of ulcerative colitis. Plevy; Scott E., et al. 435/6; 435/7.31. C12Q001/68 G01N033/569.
- ☐ 4. 5932429. 11 Apr 97; 03 Aug 99. Methods of diagnosing clinical subtypes of crohn's disease. Targan; Stephan R., et al. 435/7.24; 435/7.31 435/7.95 435/975 436/506 436/508. G01N033/564.
- ☐ 5. 5914239. 08 Nov 96; 22 Jun 99. Diagnosis of fungal infections, and a chitin-binding lectin useful in such diagnoses. Laine; Roger A.. 435/7.31; 435/34 435/35 435/7.9 436/518. G01N033/53.
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L3: Entry 9 of 35

File: USPT

Sep 19, 2000

US-PAT-NO: 6121420

DOCUMENT-IDENTIFIER: US 6121420 A

TITLE: Diagnosis of fungal infections, and a chitin-binding lectin useful in such diagnoses

DATE-ISSUED: September 19, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laine; Roger A.	Baton Rouge	LA		

US-CL-CURRENT: 530/350; 435/7.31

CLAIMS:

I claim:

1. Substantially pure chitovibrin; wherein said chitovibrin is a protein of molecular weight about 134 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; wherein said chitovibrin has affinity for chitin and for chito-oligomers dp9 and larger; wherein said chitovibrin is obtained from or is identical to a protein obtained from secretions from marine bacteria of the genus *Vibrio* induced by the presence of chitin, chitin oligomers, or cellobiose; wherein said chitovibrin has an isoelectric pH of about 3.6; wherein said chitovibrin binds chitin at an optimum pH of about 6; wherein said chitovibrin binds to chitin in aqueous solutions throughout a range of NaCl concentrations from 0 M NaCl to about 4 M NaCl; and wherein said chitovibrin has no hydrolytic activity towards chitin, towards chitin oligomers, or towards chitobiose.
2. Substantially pure chitovibrin as recited in claim 1, wherein the amino-terminal sequence of said chitovibrin is SEQ ID NO. 1.
3. Substantially pure chitovibrin as recited in claim 1, wherein said chitovibrin is obtained from or is identical to a protein obtained from secretions induced in the *Vibrio parahemolyticus* strain having accession number ATCC 27969 by the presence of chitin, chitin oligomers, or cellobiose.
4. A substantially pure polypeptide, wherein:
 - (a) said polypeptide has affinity for chitin and for chito-oligomers dp9 and larger; and said polypeptide has no hydrolytic activity towards chitin, towards chitin oligomers, or towards chitobiose; and
 - (b) said polypeptide is a fragment of chitovibrin, wherein the chitovibrin is a larger protein of molecular weight about 134 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; the chitovibrin has affinity for chitin and for chito-oligomers dp9 and larger; the chitovibrin is obtained from or is identical to a protein obtained from secretions from marine bacteria of the genus *Vibrio* induced by the presence of chitin, chitin oligomers, or cellobiose; the chitovibrin has an isoelectric pH of about 3.6; the chitovibrin binds chitin at an optimum pH of about 6; the chitovibrin binds to chitin in aqueous solutions throughout a range of NaCl concentrations from 0 M NaCl to about 4 M NaCl; and the chitovibrin has no hydrolytic activity towards chitin, towards chitin oligomers, or towards chitobiose.

5. A polypeptide as recited in claim 4, wherein said polypeptide is obtained from or is identical to a protein obtained from the proteolytic breakdown of chitovibrin by endogenous protease activity of *Vibrio parahaemolyticus*; said polypeptide has a molecular weight about 80-85 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; said polypeptide binds chitin at an optimum pH of about 6; said polypeptide binds to chitin in aqueous solutions throughout a range of NaCl concentrations from 0 M NaCl to about 4 M NaCl.



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L4: Entry 5 of 9

File: USPT

Jun 22, 1999

US-PAT-NO: 5914239

DOCUMENT-IDENTIFIER: US 5914239 A

TITLE: Diagnosis of fungal infections, and a chitin-binding lectin useful in such diagnoses

DATE-ISSUED: June 22, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Laine; Roger A.	Baton Rouge	LA		

US-CL-CURRENT: 435/7.31; 435/34, 435/35, 435/7.9, 436/518

CLAIMS:

I claim:

1. A method for detecting chitin in a sample, comprising the steps of:
 - (a) contacting the sample with a substance comprising chitovibrin; and
 - (b) inspecting the sample for the presence of chitovibrin bound to chitin, wherein bound chitovibrin indicates the presence of chitin in the sample;wherein said chitovibrin is a protein of molecular weight about 134 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; wherein said chitovibrin has affinity for chitin and for chito-oligomers dp9 and larger; wherein said chitovibrin is obtained from or is identical to a protein obtained from secretions from marine bacteria of the genus *Vibrio* induced by the presence of chitin, chitin oligomers, or cellobiose; wherein said chitovibrin has an isoelectric pH of about 3.6; wherein said chitovibrin binds chitin at an optimum pH of about 6; wherein said chitovibrin binds to chitin in aqueous solutions throughout a range of NaCl concentrations from 0 M NaCl to about 4 M NaCl; and wherein said chitovibrin has no hydrolytic activity towards chitin, towards chitin oligomers, or towards chitobiose.
2. A method as recited in claim 1, wherein said chitovibrin is conjugated to a detectable label.
3. A method as recited in claim 2, wherein the detectable label is selected from the group consisting of a radioactive material, a fluorophore, a dye, an electron-dense compound, and an enzyme.
4. A method as recited in claim 1, wherein the sample comprises a plant tissue, an agricultural product, an animal tissue, a human tissue, a contact lens, a prosthetic device, or an air filter.
5. A method as recited in claim 4, wherein said inspecting step comprises inspecting the sample for the presence of chitovibrin bound to chitin in fungal cell walls.
6. A method as recited in claim 4, wherein said inspecting step comprises inspecting the sample for the presence of chitovibrin bound to chitin in yeast bud scars.
7. A method as recited in claim 1, wherein the sample comprises an animal body

- fluid, a human body fluid, a plant fluid, potable water, or a beverage.
8. A method as recited in claim 7, wherein said inspecting step comprises inspecting the sample for the presence of chitovibrin bound to chitin in fungal cell walls.
9. A method as recited in claim 7, wherein said inspecting step comprises inspecting the sample for the presence of chitovibrin bound to chitin in yeast bud scars.
10. A method as recited in claim 1, wherein said contacting step additionally comprises contacting the sample with a reagent comprising an antibody to chitovibrin.
11. A method as recited in claim 10, wherein said reagent additionally comprises a detectable label.
12. A method as recited in claim 11, wherein the detectable label is selected from the group consisting of a radioactive material, a fluorophore, a dye, an electron-dense compound, and an enzyme.
13. A method as recited in claim 10, wherein the sample comprises a plant tissue, an agricultural product, an animal tissue, a human tissue, a contact lens, a prosthetic device, or an air filter.
14. A method as recited in claim 13, wherein said inspecting step comprises inspecting the sample for the presence of chitovibrin bound to chitin in fungal cell walls.
15. A method as recited in claim 13, wherein said inspecting step comprises inspecting the sample for the presence of chitovibrin bound to chitin in yeast bud scars.
16. A method as recited in claim 10, wherein the sample comprises an animal body fluid, a human body fluid, a plant fluid, potable water, or a beverage.
17. A method as recited in claim 16, wherein said inspecting step comprises inspecting the sample for the presence of chitovibrin bound to chitin in fungal cell walls.
18. A method as recited in claim 16, wherein said inspecting step comprises inspecting the sample for the presence of chitovibrin bound to chitin in yeast bud scars.
19. A method for detecting chitin in a sample, comprising the steps of contacting the sample with a substance comprising a polypeptide, and inspecting the sample for the presence of said polypeptide bound to chitin, wherein bound polypeptide indicates the presence of chitin in the sample; wherein:
- (a) said polypeptide has affinity for chitin and for chito-oligomers dp9 and larger; and said polypeptide has no hydrolytic activity towards chitin, towards chitin oligomers, or towards chitobiose; and
- (b) said polypeptide is obtained from or is identical to a polypeptide obtained from the proteolytic breakdown of chitovibrin by endogenous protease activity of *Vibrio parahemolyticus*; said polypeptide has a molecular weight about 80-85 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; said polypeptide binds chitin at an optimum pH of about 6; said polypeptide binds to chitin in aqueous solutions throughout a range of NaCl concentrations from 0 M NaCl to about 4 M NaCl; wherein chitovibrin is a larger protein of molecular weight about 134 kDa as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; wherein chitovibrin has affinity for chitin and for chito-oligomers dp9 and larger; wherein chitovibrin is obtained from or is identical to a protein obtained from secretions from marine bacteria of the genus *Vibrio* induced by the presence of chitin, chitin oligomers, or cellobiose; wherein

chitovibrin has an isoelectric pH of about 3.6; wherein chitovibrin binds chitin at an optimum pH of about 6; wherein chitovibrin binds to chitin in aqueous solutions throughout a range of NaCl concentrations from 0 M NaCl to about 4 M NaCl; and

wherein chitovibrin has no hydrolytic activity towards chitin, towards chitin oligomers, or towards chitobiose.

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- ☐ 11. [5187064](#). 20 Dec 88; 16 Feb 93. Monoclonal antibodies and methods for fungal pathogen detection. Petersen; Frank P., et al. [435/7.31](#); [435/34](#) [435/341](#) [435/7.9](#) [436/518](#) [436/548](#) [530/388.5](#). C07K015/02 C12Q001/04 G01N033/577.
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